REMARKS/ARGUMENTS

Claims 1-35, and 54-60 are pending. Claims 12 and 13 have been withdrawn from consideration as directed to unelected species of the invention. It is Applicants' understanding that if the elected subject matter is found to be allowable over the prior art, the search and examination will be expanded to cover other species (including those claimed in claims 12, and 13) until it includes the full scope of the generic claims. Claim 1 has been amended. Support can be found, for example, in the specification on page 23, lines 23-26. Claims 61-63 have been added. Support for claims 61-63 can be found, for example, in the specification on page 83, lines 15-19. Claim 5 has been cancelled. After entry of the present amendments, claims 1-4, 6-35, and 54-63 will be pending.

Formal matters

As of this date, Applicants have not received an initialed copy of the Information Disclosure Statements filed June 12, 2003, February 25, 2003, October 18, 2002, July 17, 2002, April 23, 2002 and July 16, 2001. Applicants therefore respectfully request the initialed Form 1449s.

Rejections under 35 U.S.C. § 103

Claims 1-4, 6-11, 14-35 and 54-60 have been rejected under 35 U.S.C. § 103 as being obvious in view of Unger et al WO 96/40285 (WO '285) in view of Ruoslahti et al., U.S. Patent No. 5,536,814 ('814 patent) and Siegel et al., U.S. Patent No. 6,086,573 ('573 patent). It is asserted in the action that WO '285 teaches a subgenus of compounds that encompass compounds of the present invention. The action acknowledges that WO '285 does not teach the use of CRGDC as the targeting agent, PEG-3400 as the hydrophilic polymer, and urokinase as the bioactive agent. The '814 patent is cited to show that CRGDC is a cyclic peptide and a suitable targeting agent and the '573 patent is cited show that the combination of a thrombolytic agent with a gaseous ultrasound contrast agent can enhance the

thrombolytic effects of a thrombolytic agent. Applicants respectfully traverse the rejection, and respectfully submit that the compounds defined in the present claims are not suggested in the teachings of the cited references, alone or in any proper combination.

The Claimed Invention

Independent Claim 1 in the present application defines targeted compounds which necessarily contain two fatty acid amide groups linked directly or through an intervening alkylene group to a tertiary carbon atom.[1] As amended herein, the *acyl groups present in the claimed compounds must have from about 16 to about 23 carbons*[2]. As will be apparent from the discussion which follows, these "di-fatty acid amide compounds" represent a particular class of compounds that are not specifically suggested in the cited prior art. Moreover, the claimed compounds provide surprising results, which are entirely unexpected in light of the disclosure of the cited references.

Discussion of the Cited Art

Although the teachings in WO '285, as set forth in Claim 136 and the description at page 82, line 7 *et seq*, represent a broad disclosure of a potentially vast genus of compounds, it does not make obvious the particular compounds of the present invention. It is respectfully submitted that there is nothing in WO '285 which would suggest to the skilled artisan the desirability of selecting the present combination of substituents from among the wide variety of disclosed substituents in WO '285, in an effort to provide Applicants' defined di-fatty acid amide compounds.

In addition to disclosing a genus encompassing an enormous number of compounds, WO '285 discloses numerous specific targeted compounds (*see, e.g.*, Examples 1 to 5, 13, 14, 44, 45, 47 to 52, 56 and 57 in WO '285). The vast majority of these specifically disclosed compounds include tertiary carbon atoms which are substituted with chemical groups other than amide groups. Indeed, none of these specifically disclosed compounds are di-fatty acid

1 The fatty acid amide groups in the compounds of formula IV are represented by the groups R^1R^2N- and R^4R^5N, where R^1 and R^4 are acyl groups of about 16 to about 23 carbons.
2 This amendment to claim 1 is supported in the original application, for example, at page 23, lines 23 to 26; and in Examples 63 and 64.

amide compounds, as defined in Applicants' claims. It is submitted respectfully that these specifically disclosed compounds can in no way render obvious Applicants' defined di-fatty acid amide compounds.

The deficiencies in the rejection are not aided by combination with the secondary references, as the '814 and '573 patents are cited merely to demonstrate respectively that CRGDC is a targeting agent and that combination of a thrombolytic agent with a gaseous ultrasound contrast agent can enhance the thrombolytic effects of the thrombolytic agent. These references contain nothing that would lead one of skill in the art to select the compounds recited in Applicants' claims.

Thus, Applicants respectfully submit that no reference has been cited which teaches or fairly suggests to one of ordinary skill in the art the subject matter of the present claims. No reference has been cited which discloses or suggests the desirability of modifying the specific compounds described in WO '285 in such a way to arrive at Applicants' compounds, nor is there anything in WO '285 to lead one of ordinary skill in the art to select the presently claimed compounds from amongst the vast number of compounds generically described in that reference. Applicants respectfully submit that the law is clear that in the absence of such a reference, there is inadequate support for an assertion by the Patent Office that the present claims are obvious. Accordingly, Applicants respectfully submit that the rejection of the claims under 35 U.S.C. § 103 be withdrawn.

Applicants' Compounds Produce Surprising Results

Furthermore, Applicants' compounds provide surprising results, which are entirely unexpected in light of the disclosure of the cited references.

It is well settled in the courts that greater than expected results are evidence of nonobviousness, *See* MPEP 716.02(a). A showing that the results were greater than those which would have been expected from the prior art to an unobvious extent, and that the results are of significant practical advantage is sufficient to overcome a *prima facie* case of obviousness. *Ex parte The NutraSweet Co.,* 19 USPQ2d 1586 (Bd. Pat. App. & Inter. 1991).

The compounds and compositions of the present invention are used, *inter alia,* to target gas-filled vesicles, including, for example, gas-filled microbubbles or liposomes, to

select tissues or cells in the body. In order for a compound of the present invention to effectively direct a vesicle to its target site, the compound is preferably inserted into the vesicle walls or membrane and remains inserted during transport (see, for example, page 19 of the specification, lines 18-28). The Applicants have discovered that the di-fatty acid amide linkage component of the claimed compounds improves the ability of the compounds to insert themselves into the vesicle membrane and remain inserted in the vesicle membrane during transport. As evidence that the compounds of the present invention provide surprising results that are greater than would have been expected from the prior art and are of a significant practical advantage, Applicants submit an abstract authored by the present inventors demonstrating that di-fatty acid amide compounds within the scope of the present claims are surprisingly better at anchoring to the vesicles thereby ensuring that the vesicles will be directed to a target site (Schumann *et al.*, Synthesis, Characterization, and Calorimetric Studies of Novel Bioconjugates for the Selective Targeting of Microbubbles to GPIIbIIIa Receptors on Vascular Thrombi, see Appendix A). These results are completely unexpected given the teachings of the cited art. The cited art does not suggest that a di-fatty acid amide component improves the ability of the compound to attach to a vesicle nor does it suggest that the length of the carbon chain influences the efficacy of the compound. Accordingly, one of skill in the art would have been surprised that the claimed compounds provide such a markedly improved result. Thus, Applicants respectfully request that the rejections under 35 U.S.C. § 103(a) be withdrawn.

Applicants believe that the foregoing constitutes a complete and full response to the Office Action of record. Accordingly, an early and favourable reconsideration of the rejections and an allowance of the claims is respectfully requested.

Date: May 13, 2004

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Registration No. 54,836

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Exhibit "A"

Synthesis, Characterization, and Calorimetric Studies of a Series of Novel Bioconjugates for the Selective Targeting of Microbubbles to GPIIbIIIa Receptors on Vascular Thrombi.

Patricia A. Schumann, Rachèl M. Quigley, Varadarajan Ramaswami, Evan C. Unger and **Terry O. Matsunaga**, ImaRx Therapeutics, Inc. Tucson, AZ

Purpose

Bioconjugate ligands have been used in our laboratory to target microbubbles to glycoprotein receptors for both diagnostic and therapeutic applications. The purpose of the bioconjugate is to: 1) anchor the molecule into the microbubble membrane, 2) provide overall flexibility for the ligand to "find" its target, and 3) provide a ligand selective for certain receptors. However, in order for ligands to effectively direct the entire microbubble to a selective receptor, the bioconjugate must remain inserted into the microbubble membrane. Failure to do so could result in "free" bioconjugates acting as competitive receptor antagonists to microbubble or liposome binding. We have conducted a calorimetric study to determine the efficiency of insertion of a series of bioconjugates that vary only in hydrocarbon tail length.

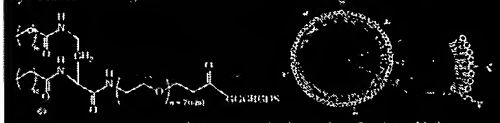

Figure 1 – Left: Structure of diacyl-diaminobutyryl-PEG-GCGRGDS. Right: Microbubble with bioconjugates attached with enlarged view showing the anchor, tether, and ligand

Materials and Methods

Synthesis and Characterization

All compounds were synthesized employing standard Fmoc coupling schemes. The diaminobutyryl-PEG-GCGRGDS was batch synthesized on Wang resin using 3 equivalents each of the suitably protected amino acid, DIC, and HOBT. Only one equivalent of Fmoc-α-amino, α-carboxy polyethylene (PEG) was used for coupling of PEG unto the terminal glycine. After the addition of the diaminobutyric acid the batch was then split for coupling of the appropriate fatty acid.

Cleavage of the bioconjugates was accomplished using standard TFA, Ethanedithiol, phenol, thioanisole, water cocktails (8.3 0.25:0.5:0.5:0.5, v:v:v:v) for 15 minutes. The cocktail was filtered, neutralized to pH = 8.0, and dialyzed exhaustively against deionized water using 1000 MWCO dialysis membranes (Spectra-Por®,Los Angeles, CA). The dialyzed filtrate was then concentrated *in vacuo* and purified by reverse-phase HPLC.

All purified samples were then characterized by amino acid analysis, NMR, and MALDI mass spectrometry.

Differential Scanning Calorimetry

Calorimetric scans were acquired on a MicroCal MC-2. Samples were prepared by mixing the bioconjugate with dipalmitoylphosphatidylcholine (DPPC). Samples were suspended in 0.9% NaCl solution followed by 6 freeze-thaw cycles. Samples were then placed in a calorimetry cell and scanned at a rate of 10° C hr^{-1} through a temperature range from 20°C – 50°C.

Initially the C18 diacyl analog in DPPC was analyzed at 1.2, 4.8 and 9.6 mole %. The 9.6 mole % mixture showed the most significant disruption of the DPPC membrane, therefore all other analogs were tested at this concentration.

Sizing

Size determinations were carried out using dynamic laser light scattering. The measurements were made at a 90° scattering angle using a 20 mW He-Ne laser at 632 nm on a Nicomp Model 370 (Particle Sizing Systems, Santa Barbara, CA). The instrument is capable of measuring particles in the range of 0.02 to 2 microns. Data were analyzed using PSS proprietary software.

Results

Reverse Phase HPLC

Reverse phase HPLC revealed an elution profile consistent with the chain length of the fatty acid moiety. Figure 2 displays the analytical HPLC profile for the distearyl-Dab-PEG-GGGRGDS after purification. Table 1 below displays the retention times for the four analogs.

Figure 2

Table 1

Retention Time for diacyl-Dab-PEG-GGGRGDS analogs

Fatty Acid Anchor	Retention time (minutes)
distearyl~	46.38
dipalmityl~	45.25
dimyristyl~	43.33
dilauryl~	37.65

NMR

300 MHz ^{1}H NMR were all consistent for a large singlet at 3.64 ppm indicative of the equivalent ethylene protons of the PEG polymeric moiety. In addition, the methylene resonances of the fatty acid moieties and the terminal methyl groups at 0.878 ppm were clearly visible. Relative integration profiles were consistent with the number of protons on the corresponding fatty acid moiety.

Amino Acid Analysis

Amino acid analysis is seen in Figure 3, is consistent with the predicted composition of the ligand. Glycine is present in a ratio of 4:1 to each arginine, aspartic acid and serine detected. Interestingly, the diaminobutyrate (Dab) is found between the retention times of phenylalanine and lysine, consistent with the fact that Dab exhibits properties similar to lysine but, due to two less methylenes, elutes slightly earlier than lysine.

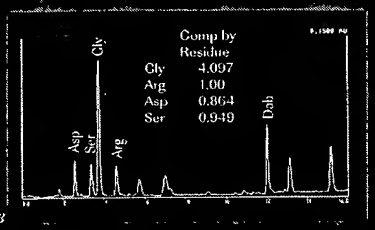

Figure 3

MALDI Mass Spectrometry

MALDI mass spectrometry results, shown in Table 2, provide excellent correlation between predicted and measured masses.

Table 2

MALDI Mass Spectrometry Data of diacyl-Dab-PEG-GGGRGDS

Diacyl group	Predicted (MH$^+$/z)	Measured (MH$^+$/z)
Distearyl (C18)	4560.62	4560.30
Dipalmityl (C16)	4557.58	4557.92
Dimyristryl (C14)	4502.48	4500.79
Dilauryl (C12)	4446.37	4446.10

Differential Scanning Calorimetry (DSC)

DSC measures the heat flow rate to a sample as a function of time or temperature. Cooperative packing of lipid membranes is exquisitely sensitive to disruptions in the packing order and can be measured by changes in the calorimetric enthalpy. Using a simple two state system, the following principles apply

A simple two-state system:

$$A \rightleftharpoons B$$

The calorimetric enthalpy is defined by:

$$\Delta H_{cal} = M \int_{t_1}^{t_2} c_{p_{ex}} dT$$

Finally, the van't Hoff equation is defined by:

$$\left(\frac{\partial \ln K}{\partial T} \right)_p = \frac{\Delta H_{vH}}{RT^2}$$

and

$$\text{Cooperative Units (CU)} = \Delta H_{vH} / \Delta H_{cal}$$

Thus, the efficiency of lipid bilayer disruption by, in this case, insertion of the lipophilic portion of the bioconjugate, can be quantitated.

Figure 4 shows calorimetric scans of A) DPPC liposome, and B) DPPC liposome mixed with 9.6 mole % the diacyl-diaminobutyryl-PEG-GGGRGDS analogs. Note the broadened peaks in B indicative of insertion of the bioconjugate into the membrane.

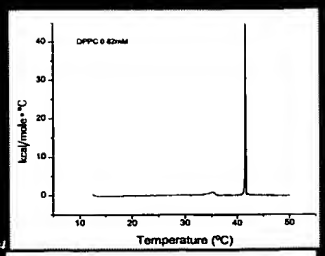

Figure 4

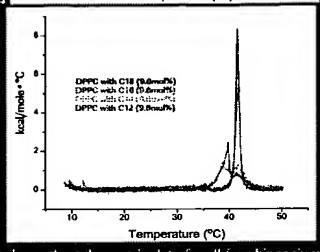

Table 3 shows the calorimetric data for all four bioconjugates differing only in hydrocarbon tail length.

Table 3

Differential Scanning Calorimetry and Sizing Data of Bioconjugates

Samples	Tm°C	$T_{1/2}$°C	ΔH Cal (Kcal/mol °C)	ΔH van't Hoff (Kcal/mol °C)	CU	Mean particle size (μm) (n=3)
DPPC	41.59	0.14	8.65	3200	304	>2
* /C18*	41.55	0.70	7.67	893	105	>2
* /C16*	41.15	1.14	6.95	487	70	0.8
* /C14*	38.62	4.29	6.16	124	20	0.06
* /C12*	38.37	2.77	5.86	143	25	0.1

* analogs

Note, the calorimetric enthalpy (ΔH Cal) decreases with shorter chain lengths, and the cooperative units follow the same trend. It should be noted that the peak width at half height increases with decreasing chain length.

Conclusions

One of the criteria for targeting microbubbles to selective receptors is that the bioconjugate carrying the targeting ligand is firmly anchored into the monolayer or bilayer membrane assembly. Hydrocarbon chain lengths play an important functional role. Calorimetric data suggests that 18 and 16 carbon chains insert into the bilayer. This is evidenced by the fact that the lipid main transition peak broadens and the cooperative unit (CU) decreases. Sizing data indicate that the large structures are still intact. Shorter chains, especially 14- and 12-carbon lengths induce significant decreases in cooperative units as well as mean particle size to the 100 nm range. The extent of disruption from the 12 and 14 carbon analogs indicate the formation of smaller structures such as liposomal, micellar or other non-lamellar assemblies. Based on these results we can conclude that in our experiments the 16 and 18 carbon analogs are more suitable for anchoring the bioconjugate to the microbubble.

Acknowledgements

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- Staff of the University of Arizona, Department of Chemistry, Mass Spec, NMR and AAA facilities.
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- Lori Nye, Creative Director, ImaRx Therapeutics, Inc.

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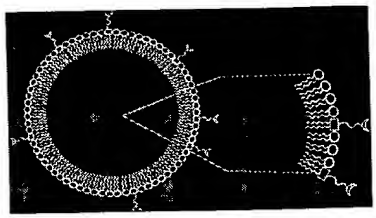

Figure 1 – microbubble with bioconjugates attached with enlarged view showing the anchor, tether, and ligand

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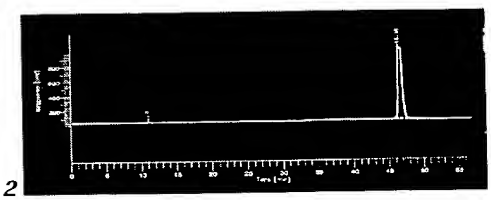

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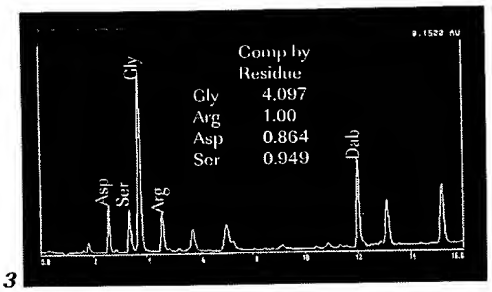

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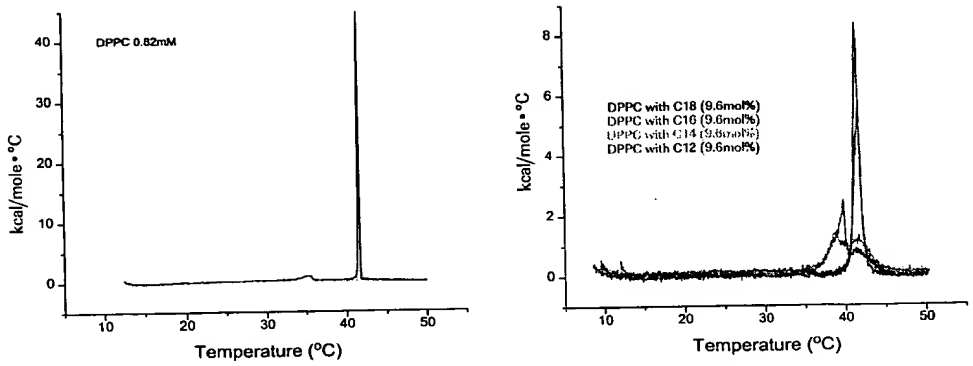

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